REMARKS

For convenience in addressing the issues raised in the Office Action, the headings used in the Action are used in this response.

PRELIMINARY AMENDMENT

Applicants acknowledge with appreciation, the entering of the Preliminary Amendment filed June 7, 1995, and the Preliminary Amendment filed (by facsimile) on May 28, 1996. Applicants also acknowledge with appreciation the treating of the amendments to claims 6, 7 and 10 as inadvertent typographical errors.

INFORMALITIES

The specification has been amended to update the status of the parent application.

It is requested that the objection to the drawings be held in abeyance pending a determination of allowable subject matter in this application.

REJECTIONS UNDER 35 USC § 112

Claim 13 has been amended to precisely recite that in the biospecific assay method of the present invention analyte concentration in a sample is determined by measuring the signal strength from an individual microparticle using a measuring means capable of reading the luminescence from an individual microparticle and comparing the signal strength from the individual microparticle with a standardization curve. In the method of the invention each of the individual microparticles is not separately

measured (refer to the discussion below relating to the objections to the specification under the first paragraph of 35 U.S.C. § 112). Claim 13, therefore, has not been amended as proposed in lines 1-3 on page 3 to recite the measuring of the signal strength from each of the individual microparticles. Claim 13 as amended is not contradictory and avoids the confusion noted in lines 5-9 on page 3 of the Action and the confusion noted in lines 11-15 on page 3 of the Action. Claim 13 has also been amended to delete the recitation "a statistically reliable measurement" thereby avoiding the confusion noted in the first paragraph on page 3 relating to such recitation.

Claims 14 and 15 have been cancelled to avoid the rejection relating thereto.

Claim 16 has been amended substantially as proposed in the Action to avoid the alleged indefiniteness relating thereto.

The alleged indefiniteness relating to claim 17 is believed to have been avoided by the amendments to claim 13.

Regarding the lack of proper antecedent basis for the multi-analyte method recited in claim 18, claim 13 has also been amended in line 3 to recite "at least one analyte to be assayed."

Finally, claim 8 has been cancelled to avoid the rejection relating thereto.

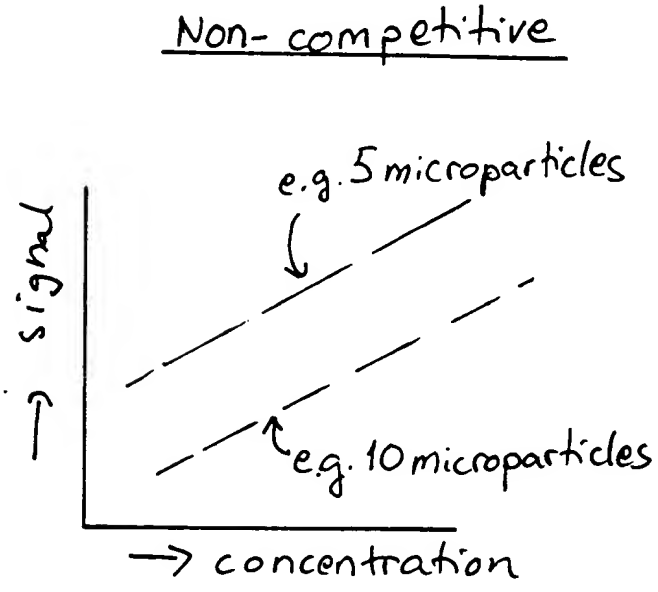
The claims as amended are believed to be definite within the meaning of the requirements of the second paragraph of 35 U.S.C. §

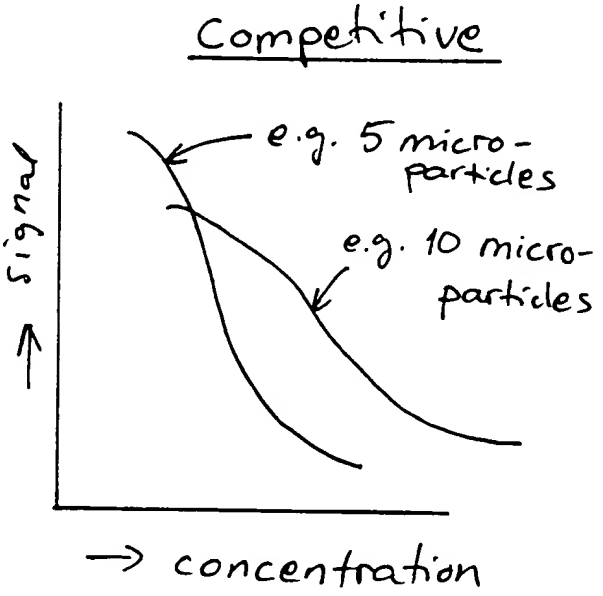
112. Removal of the §112, second paragraph, ground of rejection is respectfully requested.

[OBJECTIONS TO THE SPECIFICATION
UNDER 35 USC § 112, FIRST PARAGRAPH]

The first objection on page 5 (lines 6-9) is exactly the same as the objection raised in the Office Action dated March 23, 1994, in the parent application. The "separate measurement of each individual microparticle" is not applicants' invention. The invention is the discovery that the use of a controlled amount of microparticles and a controlled amount of sample will allow for improved measurement of the concentration of an analyte in a sample (where the samples will typically have a concentration within a certain range). The amount of microparticles and the amount of sample must be determined by experimentation using samples having a known concentration of analyte where the concentration is within a "typical" range. This experimentation is carried out during development and production. It is not assumed that the user is required or needs to do this. The fact that such experimentation is required does not make the invention indefinite and does not mean that the invention does not have a utility nor does it mean that the necessary experimentation is undue experimentation. Whether or not experimentation is "undue" depends on the nature of the experimentation and not the amount of the experimentation. Removal of this first objection is requested.

Regarding the (second) objection described on page 5, line 10, to page 6, line 2, of the Office Action, it appears that the logic of the present invention is not understood by the Office. When the assay method of the present invention is applied in either a conventionally designed non-competitive or competitive immunoassay by, for example, using two different amounts (i.e., numbers) of microparticles but otherwise identical assay conditions, the standard curves shown below are obtained.





Thus, the assay conditions are set with a suitable number of microparticles to obtain the relevant sensitivity and range which then will be repeatedly used. Consequently, there is no need for a continuous standardization in order to obtain correct results. According to the invention, the range and sensitivity are adjusted by selecting sample volume and particle number to obtain a relevant working range. The result is read from an individual microparticle (although more than one measurement of concentration using an individual microparticle may be made to ensure statistical reliability). This can be achieved very simply for any new analyte for which the assay method of the invention is to be applied.

The comments in the Action on page 5, lines 10-15, do not appear appropriate because a fractional occupancy principle is not used in the invention (refer to the comments below) and, additionally, a percentage of the labeled hapten (antigen) is bound only in the competitive assay format. The protocol for experimentation, therefore, is clear. The necessary experimentation during the development of the assay is an expected amount of experimentation and the assay method of the invention can be practiced without undue experimentation from a reading of the specification when considered in light of the prior art. Nothing further is required under the first paragraph of 35 U.S.C. § 112. Removal of the second objection is requested.

The third objection relating to the DELFIA assay (page 6, lines 3 and 4) was also raised in the March 23, 1994, Action in the parent application. In the response filed August 22, 1994, to this Action, the following comments were made in response to the objection:

> The DELFIA technology described in this invention is used as a reference procedure to quantitate the label in a suspension of microparticles. Part of the sample particles are measured individually in the time-resolved microfluorometer, in which case the fluorescence is read directly from the surface of the microparticles without the use of the DELFIA technology (no enhancement solution for dissociation of the label into solution is required). The DELFIA technology is described in the attached Chapter 16 of the book: Luminescence Immunoassay and Molecular Applications, CRC Press. DELFIA is a commercial system. Note. p. 238 of the article.

In the Office Action dated December 13, 1994, the following was noted on page 6, line 28, concerning this objection: "Withdrawn in view of Lövgren et al. reference attached to Paper #6." Withdrawal of this rejection is again appropriate.

The fourth objection relating to the "sensitive label technology" (page 6, lines 5 and 6 of the Action) was also raised in the March 23, 1994, Office Action. In response to this objection, the terminology "by the sensitive label technology used" was deleted from the claims. The Applicants note that as used in the application, the terminology refers to affinity techniques such as immunoassay, hybridizations, etc. The Office was referred to

the Ekins article described on page 3, lines 22-24, of the specification.

In the Office Action mailed December 13, 1994, the Office noted on page 7, lines 4 and 5, that this objection was "moot in view of the cancellation of the subject matter from the claimed invention". Removal of the objection as being "moot" is also believed to be in order in this application.

Regarding the fifth objection relating to the difference from routine optimization (page 6, lines 7-14, of the Action), the statement in the Action that "[h]owever, routine optimization suggests using as small an excess of solid phase reactant as possible to prevent unnecessary 'diluting' the signal of later-bound labelled reactant" is not true for particle-based assays. It is noted, relating to this objection, that in the Office Action dated December 13, 1994, the Office stated (in connection with the prior art rejection based on Soini, U.S. Patent No. 5,028,545) on page 7, lines 24-26: "...it is unclear how the claim 1 differs from routine optimization of assay parameters in a particle-based assay." In response to this statement it was noted in the response filed June 13, 1995, and is again emphasized herein, that the Soini patent does not each or suggest to a person of ordinary skill in the art that very low analyte concentrations can be assayed by **decreasing** the amount of microparticles. The person of ordinary skill in the art would not, with a reasonable expectation of

success, have been motivated to modify the method described in the Soini by **decreasing** the amount of microparticles. In assays known prior to the present invention it was normal routine to **increase** the binding surface when small amounts of analytes were to be measured. The immunoassay method of the present invention, therefore, is not a matter of routine optimization. The "optimization" method according to the present invention (the adjustment of amount of microparticles and sample volume used so as to achieve an easily detectable signal strength from an individual microparticle) is an entirely new idea which has never been described before. Withdrawal of the fifth objection is requested.

Finally, the sixth objection made in line 15 on page 6 of the Action relating to the "wet"/"dry" differentiation in Figure 1 and its relation to Example 1, the individual microparticles in Example 1 were measured in the microfluorometer either in a "dry" state, or in a "wet" state in a buffer solution. The results show that "dry" state measurement gives a somewhat higher signal than the "wet" measurements in the hCG assay used as an example to illustrate the invention. Both measurement modes provide a working standard curve for the assay. Withdrawal of the sixth objection is requested.

Removal of the objections to the specification under the first paragraph of 35 U.S.C. § 112 is believed to be in order and is respectfully solicited.

REJECTIONS UNDER 35 USC § 103

Referring, first, to the rejection of claims 6, 8, 10 and 13-18 as being unpatentable under 35 U.S.C. § 103 over Soini et al., U.S. Patent No. 5,028,545, alone, or, as necessary, further in view of either Ekins et al. (*Clinical Chemistry* 37 (11):1955-1967, 1991) or Buechler et al., U.S. Patent No. 5,089,391, it is respectfully submitted that the Soini et al. patent, taken alone or in any combination with Ekins et al. and Buechler et al., fails to support a *prima facie* case of obviousness of the assay method recited in the claims of the application (as amended).

The Soini et al. patent describes experiments based on the use of a flow cytometer where very large amounts of microparticles are used. The patent does not at all teach how immunoassays can be performed in order to obtain quantitative, sensitive and reproducible results from individually measured microparticles. The Soini et al. patent describes only a procedure to perform a multiparameter assay in a flow cytometer by recognizing batches of particles of different categories.

The Soini et al. patent does not teach or suggest to a person of ordinary skill in the art that very low analyte concentrations can be assayed by decreasing the amount of microparticles. The person of ordinary skill in the art would not, with a reasonable expectation of success, be motivated to modify the method described in the Soini et al. patent by decreasing the amount of

microparticles. In assays known prior to the present invention, it was normal routine to increase the binding surface when small amounts of analytes were to be measured.

The insufficiencies of the Soini et al. patent are not avoided by the combination with the Ekins et al. publication or the Buechler et al. patent. First, the teachings of the Ekins et al. publication cannot be properly combined with the disclosure of the Soini et al. patent. The immunoassay concept described in the Ekins et al. publication is completely different from the one described in the present invention. The Ekins et al. invention is based on his theory of fractional occupancy when a very small and limited amount of antibody is used to bind a fraction of an analyte, irrespective of sample volume, that the ratio between the total amount of antibody and the fraction of it that binds the antigen gives the answer, the amount of antigen measured. In addition, Ekins et al. proposes "compact disc" formats with a plurality of spaced-apart locations for different analytes to be measured, a format that no one so far has been able to reproduce. In the present invention, on the other hand, conventional bioaffinity reactions and immunoassay procedures are used with either a non-competitive or a competitive assay format (refer, for example, to the reference to R. Ekins on page 3, lines 22-24, of the specification). Thus the performance of the assays as such does not differ from the formats that are presently in use.

Consequently, the solid phase coating for the microparticles are carried out in batch format according to procedures well-known to experts in the art. There is no need to coat individual microparticles separately although individual particles are measured in the method. One coating alternative has already been used in the examples of the application using streptavidin as the primary binding component on the microparticles.

Attached hereto for the convenience of the Examiner is the comparison that was attached as Appendix 5 in the response dated June 13, 1995, in the parent application. It can be seen from the comparison in Appendix 5 that the method of the present invention, the Soini et al. method and the method of the Ekins et al. publication are based on entirely different assay principles. In the conventional non-competitive immunoassay, all the analyte molecules in a fixed sample volume are attached to the immobilized antibody. In the occupancy method (Ekins) only a fraction of the analyte molecules is attached to the immobilized antibody, wherein the amount of bound fraction is dependent only on the analyte concentration in the sample (according to the law of mass action) and is independent of the amount of sample.

On page 8, lines 3-13, of the Action, the Office has made certain statements relating to the teachings of the Ekins et al. publication. These statements are not accurate and appear to be based on confusion concerning immunoassay principles and theories.

Applicants note the following concerning the teachings of Ekins et al.

First, the Ekins et al. paper explains two basically different immunoassay concepts which the authors name the "Basic Immunoassay Designs" (pages 1956 and 1957) and the "Occupancy Principle" (starting on page 1957 and continuing on pages 1960-1964). The assay method of the present invention follows the basic immunoassay designs and not the fractional occupancy principle. This fact has been stressed in the prior responses in the parent application. These two basically different immunoassay principles should not and cannot be mixed, or confused.

Second, the Yalow-Bernson and the Ekins et al. theories concerning immunoassay sensitivity differ from each other. It is generally acknowledged that Ekins is correct. Both discuss the sensitivity of the "basic immunoassay designs", but the sensitivity does not change the design of the assays and, as such, does not have any influence on the method of the present invention. This should be completely clear after, for example, referring to the section "Definition of Assay Sensitivity" on pages 1955 and 1956 of Ekins et al. The description, therefore, in the Action on page 8, lines 3-13, is not believed to be relevant to the issue of *prima facie* obviousness of the method of the present invention under 35 U.S.C. § 103.

The teachings of Buechler et al. also do not satisfy the insufficiencies of the Soini et al. patent. The Buechler et al. patent describes a modification of a competitive ligand-receptor assay ("receptor" being, for example, an antibody) with assay conditions optimized to give an analyte threshold concentration. The assay is performed under conditions where all of the labelled ligand analog is bound to the receptor in the absence of the sample analyte. In the presence of increasing concentrations of sample analyte the ligand analog is released from the receptor within a narrow analyte concentration range, named the threshold concentration, and a positive signal can be detected. After binding, the reaction terminal solid phase is introduced to capture and detect the released ligand analog. The method of the present invention, on the other hand, utilizes a technique where a predetermined, clinically relevant range of an analyte is measured from a surface of a single microparticle. The basic ideas and optimizations needed to reach the goal of Buechler et al. and that of the present invention are entirely different.

For the above reasons, a person of ordinary skill in the art is not provided with a suggestion or motive to modify the method of the Soini et al. patent so as to obtain an assay method as now recited in the claims of the application. Removal of the 35 U.S.C. § 103 ground of rejection that was applied in the Action to claims

6, 8, 10 and 13-18 is believed to be in order and is respectfully solicited.

The final ground of rejection is of claim 7 as being unpatentable under 35 U.S.C. § 103 over the references described above taken further in view of Bush et al., Analytical Biochemistry 202 146-151, 1992. The Bush et al. publication, however, does not concern measuring analyte concentrations from individual microparticles. In Bush et al. a suspension of microparticles in a batch form is used to collect PCR amplified DNA target and the target collected is measured with a labelled probe using time-resolved fluorometry. The signal from the total microparticle suspensions is measured in the instrument. Consequently, the Bush et al. publication merely describes a conventional use of a microparticle suspension to read the result from a PCR amplification-based DNA hybridization assay. Removal of the 35 U.S.C. § 103 ground of rejection of claim 7 is also believed to be in order and is respectfully solicited.

The foregoing is believed to be a complete and proper response to the Office Action dated June 10, 1996, and is believed to place the application in condition for allowance. If, however, minor issues remain which can be resolved by means of a personal telephone interview, the Examiner is respectfully requested to contact the undersigned attorney at the telephone number indicated below.

In the event that this paper is not considered to be timely filed, applicants hereby petition for an appropriate extension of time. The fee for any such extension may be charged to our Deposit Account No. 01-0305.

In the event any additional fees are required, please also charge our Deposit Account No. 01-0305.

Respectfully submitted,

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Appendix

Lövgren et al.

A. Non-competitive assays

1. Solid phase as well as labelled antibody in excess over analyte.

2. As large amount of the analyte as possible is collected by the solid phase.

3. The assay can be performed in either one or two steps.

4. An increase in sensitivity is achieved by collecting the analyte on as small number of particles as possible.

5. Measurement of high analyte concentration is achieved by increasing the number particles used per assay.

6. The number of particles is adjusted to measure as large as possible analyte range with required sensitivity.

B. Competitive assays

1. A suitable amount (depends on assay sensitivity) of analyte specific antibody is bound to the solid phase.

2. In the incubation step the analyte in the sample and a suitable amount of labelled analyte compete during the binding to the solid phase antibody.

3. In a sensitive competitive assay as small as posibly number of particles is used.

4. After separation the amount of labelled analyte bound to the solid phase is measured.

Ekins et al.

A. Non-competitive assays

1. Concentration of solid phase antibody has to be low(<0.05/K).

2. In the first incubation step a fraction of the analyte is collected by the solid phase antibody.

3. The amount of bound analyte is measured in the second incubation by the addition of labelled antibody (fractional occupancy).

4. The fractional occupancy can be measured when the solid phase antibody is also labelled.

5. The fractional occupancy is independent of sample volume and the amount of solid phase antibody when the bound analyte concentration is small enough not to affect the concentration of free analyte. Consequently a low solid phase antibody concentration (<0.05/K) has to be used.

B. "Competitive" assays

1. A low concentration (<0.05/K) of analyte specific antibody is bound to the solid phase.

2. In the first incubation step a fraction of the analyte is collected by the solid phase antibody.

3. In a second incubation step a labelled analyte is bound to the free binding sites on the solid phase antibody.

4. The fractional occupancy can be measured when the solid phase antibody is also labelled.

5. The fractional occupancy is independent of sample volume and the amount of solid phase antibody when the bound analyte concentration is small enough not to affect the concentration of free analyte. Consequently a low solid phase antibody concentration (<0.05/K) has to be used.